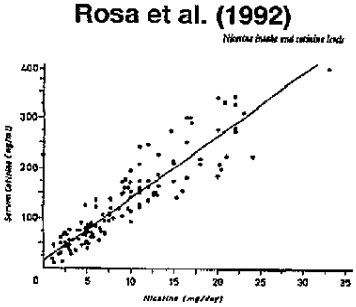
Rosa, et al., 1992

- 125 smokers who had smoked preferred brand for >24 weeks (approx. 11 cigt./d) in 4 cigarette 'tar'/nicotine groups
- Serum cotinine measured 8 h after last cigarette (HPLC)

Rosa, M., Pacifici, R., Allieri, L., Pichini, S., Ottaviani, G., Zucarro, P., How the steady-state cotinine concentration in cigarette smokers is directly related to nicotine intake, Clin. Pharm. Ther., 52: 324-329 (1992)

Rosa et al. (1992)



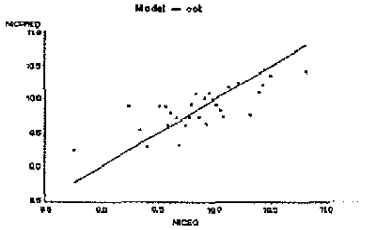
Distribution of Nicotine and Metabolites In Human Urine

Compound	%(a)	% Sum	%(b)	% Sum
3HC	35	35	36	36
COT-G	17	52	14	50
COT	13	65	9	59
NIC	10	75	9	68
3HC-G	9	84	3	91
NIC-G	3	87	5	96
NNO	7	94	3	99
ONC	4	98	1	100
DMC	2	100	N.D.	
(Smokers) N =		*11*	*91*	

a. Byrd GD, Robinson JH, Caldwell WS, deBethizy JD, Nicotine uptake and metabolism in smokers. Paper presented at the 48th Tobacco Chemists' Research Conference, Greensboro, NC. September 26-28, 1994. RSD values calculated to be 21-75%

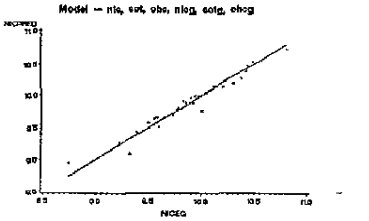
b. Abdersson G, Vale ED, Ourvell M, *The influence of cigarette consumption and smoking machine yields of tar and nicotine on the nicotine uptake and oral mucosal lesions in smokers.* J Oral Pathol Med 1997;26:117-123. RSD values calculated to be 25-100%

Urinary Cotinine Model



Boswell C, Curvall,M, Elswick Jr.,RK, Layden,D, *Modeling nicotine intake in smokers and snuff users using biological fluid nicotine metabolites*, Submitted for Publication.

Urinary Nic, Cot, 3OH & Conjugates



Boswell C, Curvall,M, Elswick Jr.,RK, Layden,D, *Modeling nicotine intake in smokers and snuff users using biological fluid nicotine metabolites*, Submitted for Publication.

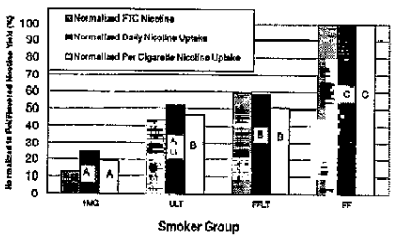
Byrd, et al., 1995

- 33 smokers of their preferred brand (approx. 35 cigt./d) in 4 cigarette 'tar' groups
- Urinary nicotine, cotinine, 3-OH-cotinine, and their glucuronide conjugates, and nicotine-N'-oxide*, cotinine-N-oxide*, demethylcotinine * measured in 24-h samples (LC/GC-MS)

Byrd, G. D., Robinson, J. H., Caldwell, W. S., deSethley, J. O. Comparison of measured and FTC-predicted nicotine uptake in smokers, Psychopharmacology 122: 95-103 (1995).

* Not determined for the smokers of 1MG cigarettes.

Data Extracted from Byrd (1995)



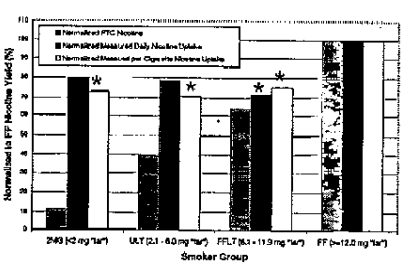
Byrd, et al., 1998

- 72 smokers who had smoked their preferred brand >6 months (approx. 33 cigt./d) in 4 cigarette 'tar'/nicotine groups
- Urinary nicotine, cotinine, 3-OH-cotinine, nicotine-N'-oxide, cotinine-N-oxide, demethylcotinine measured in three 24-h samples (LC-MS); salivary cotinine prior to last meal of day (RIA)

Byrd, G. D., Davis, R.A., Goldman, M. S., Robinson, J. H., and Bobbitt, J. D., A further study of FTC yield and nicotine absorption in smokers, Psychopharmacology 106:331-339 (1992)

Data Extracted from Byrd (1998)

(* Indicates different from FF)



Dietary Intake is Not an Issue

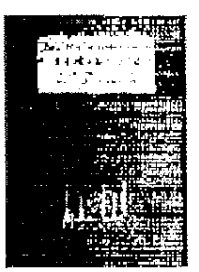
- Philip Morris sponsored a project that determined that dietary nicotine intake is not an issue in using nicotine metabolites as biomarkers for nicotine uptake either by smoking or ETS exposure.

Siegmund B, Leitner E, Pfannhauser W, Development of a simple sample preparation technique for the gas chromatographic-mass spectrometric determination of nicotine in edible nightshades (*Solanaceae*). J Chromatogr A 840(2): 249-256 (1999).

Siegmund B, Leitner E, Pfannhauser W, Determination of the nicotine content of various edible nightshades (*Solanaceae*) and their products and estimation of the associated dietary nicotine intake. J Agric Food Chem 47(8): 3113-3120 (1999).

What We Learned

- Results from population studies are not consistent between studies, even within the same laboratory
- Previous studies can be used to guide the experimental design process for future studies
- A majority of population studies have used nicotine and/or its metabolites



Biomarker Challenges

- Uniqueness or nearly so for tobacco smoke
- Representative of particulate and gas phase
- Representative of health-relevant constituents
- Understanding of constituent metabolism
- Concentration reflects uptake of cigarette smoke constituent(s)
- Based on NRC guidelines of 1986

Benowitz, N.L., Biomarkers of environmental tobacco smoke exposure, Environmental Health Perspectives, 107(2): 349–355 (1999)

Examples of Potential Smoke Constituents or their Metabolites as Biomarkers of Exposure

Particulate Phase

- Nicotine Metabolites (More than just cotinine)
- 4-aminobiphenyl (adducts)
- benzo[a]pyrene (adducts)
- PAH (adducts to plasma albumin)
- TSNA related biomarkers
- solanesol
- metals

Gas Phase

- carbon monoxide (COHb)
- cyanide (thiocyanate)
- acetonitrile
- aldehyde metabolites
- 2,5-dimethylfuran

Potential Improvements

- Potential improvements in experimental design include
  - » smoke constituent, biomarker and biologic medium studied
  - » size and diversity of smoker population
    - geographic, ethnic, age, and gender demographics
    - sufficient number of participants to ensure statistical power
  - » smoker compliance with test procedures
  - » minimize impact on subject smoking behaviors determine relationships between cigarette parameters and total exposure results

Goal of "Total Exposure" Project

Using biomarkers, determine the uptake of vapor phase and particulate smoke constituents for smokers of cigarettes with a range of yields of these constituents to provide a baseline for future cigarette-design related studies.

Collaboration Opportunity

- What can Philip Morris contribute to this study?
  - » Knowledge of cigarette design, construction and testing
  - » Expertise in smoke composition and analysis
  - » Knowledge of potential biomarkers and their analysis
  - » Awareness of the results of previous research in the area
- What can other experts contribute to this study?
  - » Input on identification and aid to prioritize health-relevant smoke constituents and their biomarkers
    - ◦ especially gas/vapor phase components
  - » population study methodologies

SUMMARY

The "Total Exposure" Project will contribute to improved understanding of the exposure of smokers to cigarette smoke constituents and establish a baseline for monitoring the impact of new cigarette designs on smokers' exposure.